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THE EFFECT OF 2-DEOXY-D-GLUCOSE AND D-GLUCOSE ON THE EFFERENT DISCHARGE RATE OF SYMPATHETIC NERVES

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SUMMARY

1. Efferent discharges were recorded from nerve filaments dissected from the adrenal and renal nerves in the rabbit.

2. An increase in discharge rate was observed in the adrenal nerve filaments following I.V. administration of 2-deoxy-D-glucose (2-DG). No change in discharge rate after 2-DG infusion was observed in the renal nerve filaments.

3. A decrease in discharge rate of the adrenal nerve filaments was observed after I.V. injection of glucose, but there was no change in the activity of renal nerve filaments.

4. Transection of the spinal cord abolished the adrenal nerve response to the systemic administration of 2-DG and glucose.

5. It is suggested that there might be a pathway from the hypothalamic area to the adrenal nerve cells of the spinal cord, but not to the renal nerve cells, through which activity of the adrenal nerve might be changed in response to 2-DG and glucose infusion.

INTRODUCTION

A marked hyperglycaemia in response to the systemic administration of a glucose analogue, 2-deoxy-D-glucose (2-DG), in rats and monkeys was reported by Smith & Epstein (1969). The administration of 2-DG to rats also causes an increased rate of secretion of adrenaline with consequent hyperglycaemia. It has been suggested that 2-DG interferes with the utilization of glucose by the brain, and that this interference activates the release of adrenaline (Hökfelt & Bydgeman, 1961).

Another glucose analogue, 3-*O*-methyl-D-glucose (3-*O*MG), was also

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found to cause a marked increase in blood glucose concentration in rats. The increase in blood glucose concentration can be accounted for by an increase in the rate of secretion of adrenaline, since the increase in blood glucose can be prevented by interruption of the central nervous connexions of the adrenal medulla (Himsworth, 1968).

These reports suggest the possibility of an increase in the efferent discharge rate of the nerves supplying the adrenal gland, following the administration of 2-DG. The present study deals mainly with the effect of 2-DG and D-glucose on the firing rate of sympathetic nerves innervating the adrenal gland (adrenal nerves) and the kidney (renal nerves).

METHODS

Twenty-four adult rabbits of both sexes were used. Food, but not water, was removed from the cages on the afternoon before an experiment. After the animal was anaesthetized with sodium pentobarbitone (Nembutal, 25 mg/kg, I.V.), the trachea was intubated. A small amount of urethane (25%, 3–5 ml.) was injected I.V. to prolong anaesthesia. A polyethylene catheter was inserted into the left jugular vein for the administration of drugs. In some animals another catheter was inserted into the cardiac end of the left carotid artery to monitor blood pressure. In other animals the e.c.g. was recorded to monitor heart rate. 2-Deoxy-D-glucose (Calbiochem), D-glucose (Nutritional Biochem Corp.) and D-mannose (Calbiochem) were dissolved in 2 ml. distilled water before use.

The small nerves from the coeliac ganglion to the left adrenal gland (adrenal nerves) were located and isolated under a dissection microscope (× 25). Nerve filaments were also dissected from the left renal nerves. The central cut end of the nerve filament was placed on a pair of silver wire electrodes and immersed in liquid paraffin (Fig. 1). Efferent nerve activity was amplified by means of a condenser-coupled differential amplifier, monitored by an oscilloscope, and stored on magnetic tape. All analysis of nervous activity was performed after conversion of the raw data to standard pulses by a window discriminator, which picked out discharges from background noise. To study the time course of the discharge rate an integrator (rate-meter) with a reset time of 5 or 10 sec was used. The discharge rate was displayed on a polygraph record (Brush) along with the efferent nerve signals.

RESULTS

2-DG effect on unitary efferent discharges

Single unit discharges were recorded from a small nerve filament dissected from a bundle of the adrenal nerve. Before the administration of 2-DG the discharge rate was about 1·4 impulses/sec (Fig. 2, upper trace). Following I.V. injection of 2-DG (150 mg/kg) the discharge rate showed a rapid increase after about 2 min. Five min after the injection, the discharge frequency reached a peak of 5·7 impulses/sec (Fig. 2, lower trace).

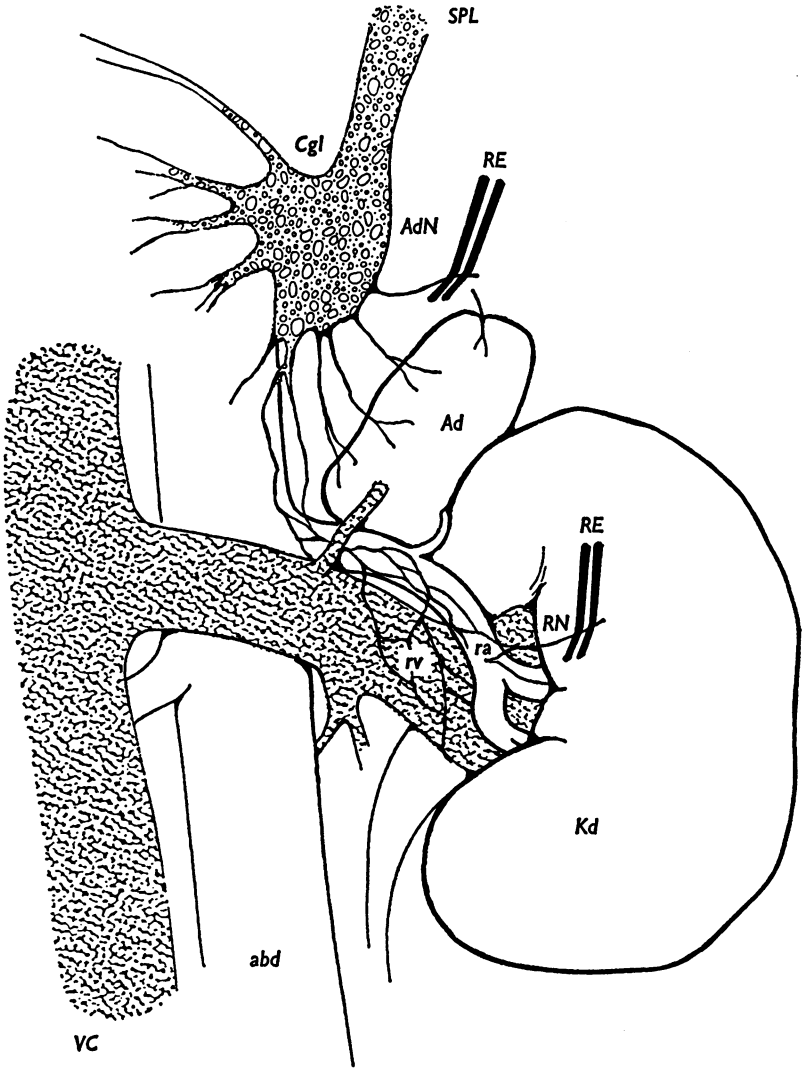

Fig. 1. Schematic diagram of the preparation showing adrenal and renal nerves used for recording. *RE*, recording electrodes; *SPL*, left splanchnic nerve; *Cgl*, coeliac ganglion; *Ad*, left adrenal gland; *AdN*, splanchnic nerve branches innervating left adrenal gland (adrenal nerves); *RN*, renal nerves; *Kd*, left kidney; *ra*, renal artery; *rv*, renal vein; *abd*, abdominal aorta; *VC*, vena cava.

Fig. 2. Effect of 2-DG injection on efferent impulses recorded from an adrenal nerve filament. Single unit discharges were recorded from the filament. Upper trace, efferent impulses recorded before injection. Lower trace, efferent impulses recorded 5 min after 2-DG injection (150 mg/kg).

Time course of 2-DG effect

The time course of changes in efferent discharge rate following administration of 2-DG was studied in adrenal nerve filaments. Recordings were made on multi-unit nerve filaments. Intravenous administration of 2-DG 50 mg/kg caused a rapid increase in discharge rate in an adrenal nerve filament about 2 min after the injection. Four min after the injection the discharge reached its peak, then slowly decreased until it returned approximately to 2-DG base line level about 23 min after the injection. An I.V. infusion of 2-DG 100 mg/kg about 10 min after the first injection also caused a rapid increase in discharge rate, starting about 4 min after this second injection. The discharge rate reached its peak 1 min later (5 min after injection), then slowly returned to the previous level about 35 min after the second injection. The peak discharge rate reached following the second injection was about 1·5 times higher than that following the first injection. The discharge rate curve also showed small fluctuations (Fig. 3, top). In another rabbit 2-DG doses of 75 and 150 mg/kg were injected successively. The interval between the injections was about 30 min. Similar results to those found with doses of 50 and 100 mg/kg were observed with this preparation (Fig. 3, bottom). Two successive injections of the same amount of 2-DG (150 mg/kg), with an interval of about 1 hr, resulted in very similar increases in discharge rate following each injection.

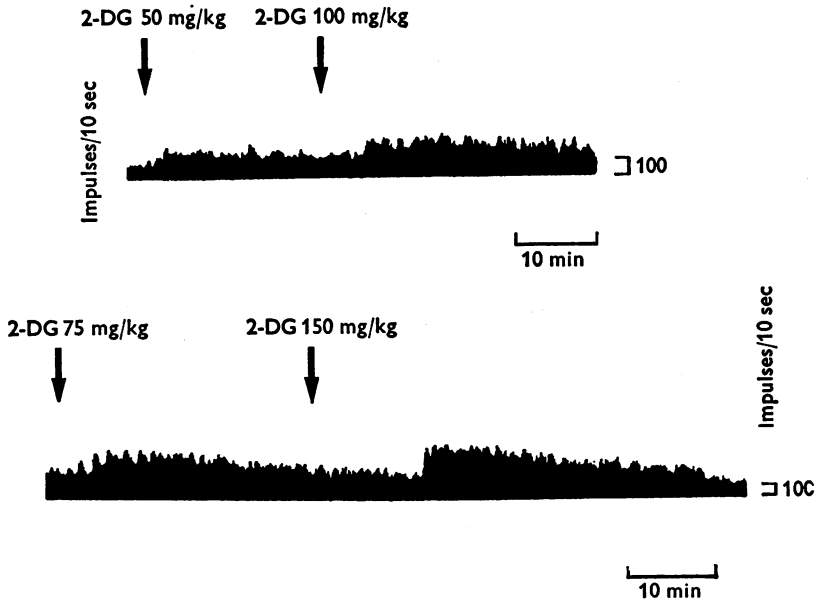

Fig. 3. Effect of 2-DG injection on efferent discharge rate of adrenal nerve. Upper and lower traces show the time course of discharge rate. Arrows show the time of 2-DG injection. First and second arrow in upper Figure indicate 2-DG, 50 and 100 mg/kg, respectively. First and second arrow in lower Figure indicate 75 and 150 mg/kg, respectively.

2-DG, mannose and glucose effects on discharge rate

Recordings were made on multi-unit nerve preparations. Intravenous administration of 2-DG (150 mg/kg) caused a rapid increase in discharge rate recorded from an adrenal nerve filament (Fig. 4, upper trace). In this preparation fluctuation in discharge rate was much more prominent than that seen in Fig. 3. The same amount of mannose (150 mg/kg), however, caused no remarkable change in discharge rate (Fig. 4, upper trace). On the other hand, I.V. injection of glucose (150 mg/kg) caused a slight decrease in discharge rate 1 min after the injection.

No change in the rate of discharge recorded from renal nerve filament (except for small changes at the time of injection) was observed following I.V. injection of 2-DG, mannose and glucose, 150 mg/kg each (Fig. 4, lower trace).

Glucose effect on discharge rate

Glucose, 60, 100 and 150 mg/kg, was successively injected I.V. and the effect on discharge rate of adrenal and renal nerve filaments was studied. In the adrenal nerve successive injections of glucose caused a stepwise

decrease in the discharge rate. Glucose 50 mg/kg caused a slight decrease 3 min after the injection, while doses of 100 and 150 mg/kg caused remarkable decreases in the discharge rate which had shown no recovery after 25 min (Fig. 5). Observations on adrenal nerve filaments in three other preparations showed similar results.

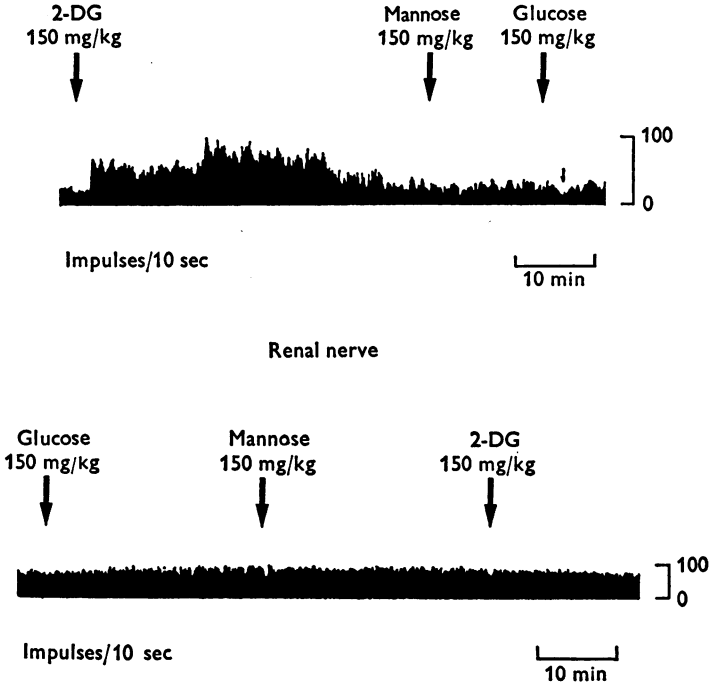

Fig. 4. Effect of 2-DG, mannose and glucose on efferent discharge rate of adrenal nerve and renal nerve. Upper trace, discharge rate recorded from an adrenal nerve filament. Left arrow shows the time of injection of 2-DG 150 mg/kg. Middle arrow shows that of mannose 150 mg/kg. Right arrow shows that of glucose 150 mg/kg. A small arrow on the upper trace shows fall in the discharge rate due to glucose administration. Lower trace, discharge rate recorded from a renal nerve filament. Arrows from left to right show the times of injection of glucose 150 mg/kg, mannose 150 mg/kg, and 2-DG 150 mg/kg, respectively.

The same amounts of glucose (50, 100 and 150 mg/kg) caused no change in the discharge rate in renal nerve filaments. Observations were made on four renal nerve filaments, and all of them showed similar results (Fig. 5).

Table 1 shows the number of observations made on the effects of 2-DG, glucose and mannose on the activity of adrenal and renal nerves.

Heart rate after the 2-DG administration showed no significant change. In those cases in which blood pressure was measured, the values fell by

26, 21 and 18% following the injection of 2-DG, glucose, and mannose, respectively. The fall in blood pressure may be explained by the fact that the tonicity of the injectates, administered at 150 mg/kg in 2 ml. distilled water, was several times higher than that of blood plasma (100 mg glucose in 2 ml. distilled water being approximately isotonic with blood plasma).

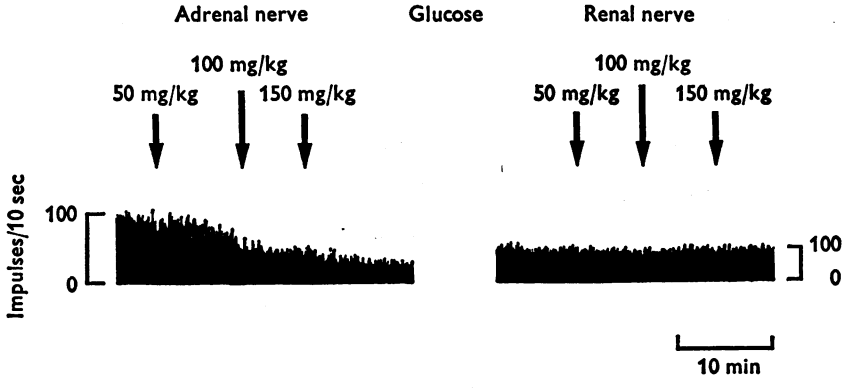

Fig. 5. Effect of glucose on efferent discharge rate of adrenal nerve and renal nerve. Left, discharge rate recorded from an adrenal nerve filament. Arrows from left to right show the times of injection of glucose 50, 100 and 150 mg/kg, respectively. Right, discharge rate recorded from a renal nerve filament. Arrows from left to right show glucose injections of 50, 100 and 150 mg/kg.

TABLE 1. Numbers of recordings and observations made and numbers of adrenal and renal nerve bundles sampled under conditions of 2-DG, glucose, and mannose infusion

	2-DG	Glucose	Mannose
Adrenal nerve activity			
Total number of recordings	17	20	4
Observations	↑ 16	↓ 19	→3
	→1	→1	↓1
Nerve bundles	8	5	2
Renal nerve activity			
Total number of recordings	8	10	3
Observations	→8	→10	→3
Nerve bundles	8	3	3

Nerve activity: ↑, increase; ↓, decrease; →, no change.

Additional experimentation has shown that there are no significant differences in the degree to which blood pressure is lowered following administration of the individual sugars at the same dosage used in the present experiment (150 mg/kg).

Dose vs. *discharge rate*

Discharge rates recorded from multi-unit preparations dissected from the adrenal nerve were plotted against the amount of glucose injected I.V. (Fig. 6, left). Observations were made in three nerve filaments. To calculate the mean discharge rate, the number of spikes in a 1 sec period was averaged over 40 sec. In one adrenal nerve filament the mean discharge

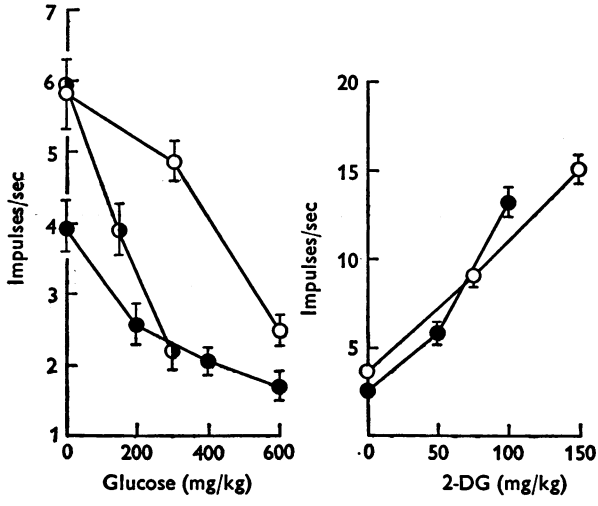

Fig. 6. Relationship between the amount of glucose or 2-DG injected I.V. and efferent discharge rate of adrenal nerve. Left graph: efferent discharge rates (ordinate) were plotted against the amount of glucose (abscissa). Observations were made on three nerve filaments. Right graph: efferent discharge rates (ordinate) were plotted against the amount of 2-DG (abscissa). Observations were made on two filaments. Each point shows mean value ± S.E. of mean (n = 40).

rate before glucose application was 3·97 impulses/sec. The discharge rate for glucose 200 mg/kg infused I.V. was 2·55 impulses/sec; the discharge rate for glucose 400 mg/kg was 2·17 impulses/sec; and that for glucose 600 mg/kg was 1·70 impulses/sec. These observations on three nerve filaments showed that when the amount of glucose was larger, the discharge rate was lower.

To examine the effect of 2-DG, observations were made on two adrenal nerve filaments. In one adrenal nerve filament the mean discharge rate before 2-DG application was 2·62 impulses/sec. The mean rate increased to 5·17 impulses/sec after the application of 2-DG 50 mg/kg. After administration of 2-DG 100 mg/kg mean discharge rate was 13·25 impulses/sec during its maximal period. It was observed in these two preparations that

the largest amount of 2-DG caused the highest discharge rate in the adrenal nerve (Fig. 6, right).

The effect of transection of the spinal cord

Four animals were used to study the effect of transection of the spinal cord on the 2-DG effect on discharge rate of the adrenal nerve. The spinal transection procedure involved initially placing the animal in a prone position for the exposure of the spinal cord at the level of T5. The animal was then placed on its right side, left side up, to make the abdominal incision and the preparation described previously. The spinal transection

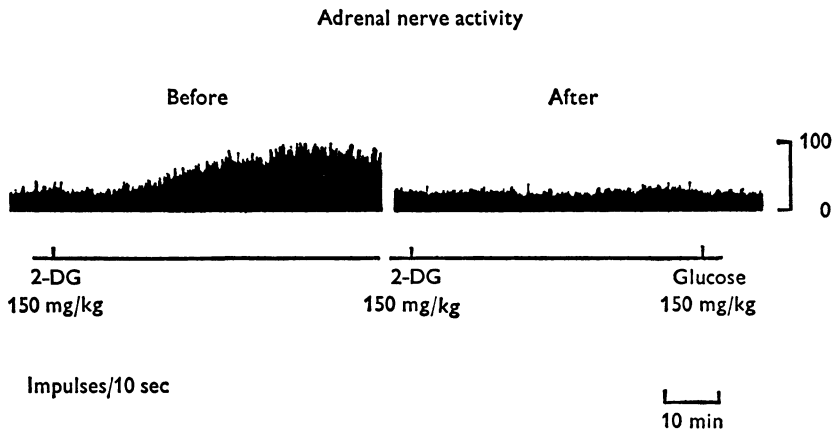

Fig. 7. Effect of spinal transection on 2-DG response. Upper traces show the efferent discharge rate. Lower traces show signals indicating the time of 2-DG (150 mg/kg) and glucose (150 mg/kg) administrations. Left Figure, before spinal transection. Right Figure, after spinal transection at the level of T5.

was made when required with a small knife while the animal remained on its right side. Before the transection 2-DG 150 mg/kg caused a remarkable increase in discharge rate (Fig. 7, left). After the spinal transection at the level of T5, 2-DG 150 mg/kg caused no increase in discharge rate. The same amount of glucose injection also caused no decrease in discharge rate. The fact that no change was found in the level of resting discharge rate before and after the transection suggests that spinal shock was not the cause of the lack of response to 2-DG and glucose (Fig. 7, right).

Competitive effects of 2-DG and glucose

Large glucose injections (600 mg/kg) reduced the discharge rate of an adrenal nerve filament by one-third. An injection of 2-DG 150 mg/kg in this condition, however, caused no remarkable increase in discharge rate

with the exception of three short bursts of firing (Fig. 8, upper trace). The same type of experiment conducted in another rabbit showed similar results.

On the other hand, the remarkable increase in discharge rate due to 2-DG infusion (150 mg/kg) could not be abolished by a large glucose injection (500 mg/kg) at the peak of discharge rate (Fig. 8, lower trace). Similar results were observed on two other rabbits.

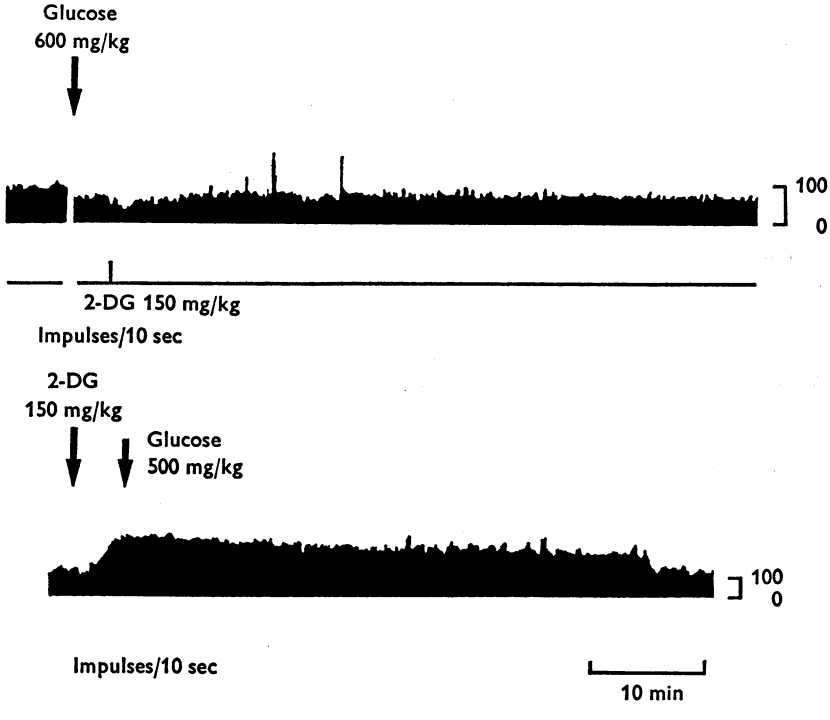

Fig. 8. Competitive effect between 2-DG and glucose. Upper Figure: an arrow shows the time of glucose injection (600 mg/kg), and a signal in the lower trace shows the time of 2-DG injection (150 mg/kg). Lower Figure: first arrow shows the time of 2-DG injection (150 mg/kg), and second arrow shows the time of glucose injection (500 mg/kg).

DISCUSSION

There are reports of a hyperglycaemic response following the systemic administration of 2-DG in the monkey and rat (Smith & Epstein, 1969) and the rabbit (Sakata, Hayano & Solviter, 1963). Since the hyperglycaemic response due to another glucose analogue (3-*O*MG) has been found to be prevented by interruption of central connexions of the adrenal

medulla (Himsworth, 1968), it was considered possible that the sympathetic nerves were also involved in the 2-DG response.

The present study showed an increase in efferent discharge rate of adrenal nerve filaments following systemic administration of 2-DG. Previous studies have shown that such an increase in the discharge must increase the rate of release of adrenaline from the adrenal medulla. The hyperglycaemic response due to 2-DG can be explained in this light (Hökfelt & Bydgeman, 1961).

The present study shows that the increase in activity in response to 2-DG administration was observed in the adrenal nerve but not in the renal nerve. The results of the experiments on spinal transection indicate that the pathway from the brain to the adrenal nerve cells in the spinal cord may play a role in activating these cells in response to 2-DG. It is unlikely that the adrenal nerve cells in the spinal cord have a direct sensitivity for 2-DG because no activation was observed following 2-DG infusion after transection of the cord.

It is known that 2-DG is a specific inhibitor for intracellular glucose utilization in the brain, muscle and liver (Brown, 1962). There are regional differences in the effect of 2-DG. It decreases glucose utilization in the brain much more than in striated muscle (Woodward & Hudson, 1954). Further, Sakata *et al.* (1963) reported that intracarotid application of 2-DG is more effective than I.V. infusion in producing hyperglycaemia. These findings suggest the existence of areas sensitive to 2-DG in the brain. It was reported by Müller, Cocchi & Forni (1971) that the hyperglycaemic effect of 2-DG injected into the lateral ventricle is greater than the effect found when 2-DG is applied systemically, suggesting the existence of a central site for the action of this drug.

The ventromedial nucleus (VMH) and lateral nucleus (LH) in the hypothalamus are known as the centres regulating feeding behaviour. It was reported that direct injection of 2-DG 1 μg into the LH in the rat induced food intake (Balagura & Kanner, 1971). The electrical stimulation of this area is effective in producing hyperglycaemia, which is diminished by adrenal demedullation (Booth, Coons & Miller, 1969). Since the rise of plasma glucose following systemic administration of 3-*O*MG is prevented by previous infiltration with lignocaine of the lateral hypothalamic areas, Himsworth (1970) has stated that there exists in the lateral hypothalamic area a chemoreceptor which controls the release of adrenaline. On the other hand, Müller *et al.* (1971) have suggested that the VMH might be sensitive to 2-DG. Relative to this study Frohman & Bernardis (1971) reported that electrical stimulation of the VMH was followed by a rapid rise in plasma glucose levels. Desiraju, Banerjee & Anand (1968) reported that the firing frequency of units recorded from the VMH was decreased

following intracarotid infusion of 2-DG, while that from the LH was increased. Oomura (1973) mentioned that direct application of 3-*O*MG to the VMH neurones produces a depression of activity in a large number of neurones, while direct application to the LH causes an increase in activity in LH neurones. These findings suggest that the activation of LH neurones or suppression of VMH neurones may cause an increase in activity of the adrenal nerve cells through a pathway in the spinal cord.

It has been reported that systemic administration, as well as direct application of glucose, increases the discharge rate of VMH neurones and decreases that of LH neurones (Brown & Melzack, 1969; Oomura, Kimura, Ooyama, Maeno, Iki & Kuniyoshi, 1964). The depressive effect of glucose infusion on the activity of adrenal nerve cells might, therefore, be initiated by these hypothalamic neurones and mediated through a pathway to the adrenal nerve cells in the spinal cord.

The experimental results reported here suggest that it is unlikely that there is a pathway to the renal nerve cells from the hypothalamic area related to 2-DG and glucose responses.

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